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Melanocortin receptors in leptin effects

Leptin acts on the central nervous system to cause a reduction in food intake and body weight^{1,2}. The melanocortin system in the brain is also implicated in energy homeostasis, with agonists of the melanocortin-4 (MC4) receptor reducing food intake³ and targeted mutation of the MC4 receptor causing obesity⁴. We now show that MC4 receptor signalling is an important mediator of leptin's effects on food intake and body weight, demonstrating a link between the two systems.

Leptin receptors are on proopiomelanocortin (POMC) neurons in the arcuate nucleus⁵ that synthesize melanocortins, therefore it seemed likely that leptin-induced reductions in food intake might be mediated by the melanocortin system. To test this hypothesis, we determined whether pharmacological blockade of MC4 receptors attenuates the ability of leptin to reduce food intake or body weight. Because leptin also activates the hypothalamic paraventricular nucleus (PVN), as measured by c-Fos expression, we also wished to determine whether this response involves melanocortin receptors.

We injected male Long-Evans rats with the non-selective MC4 receptor antagonist SHU9119 into the third cerebral ventricle (i3vt). There was a significant increase in food intake 4 and 24 h after administration of 1 nmol SHU9119, whereas 0.5 nmol altered neither food intake nor body weight at any time point. We therefore pretreated rats with 0.5 nmol of SHU9119 or vehicle i3vt before an i3vt injection of 3.5 µg of recombinant leptin or its vehicle (see ref. 1 for details). Consistent with previous findings¹, leptin alone significantly reduced food intake after 4 h (−54%) and after 24 h (−46%, $P < 0.05$; Fig. 1a), and reduced body weight after 24 h (−18.6 g, $P < 0.05$). However, when these same animals were pretreated with SHU9119, leptin did not affect food intake or body weight.

To assess the specificity of SHU9119 to reverse anorexia induced by leptin, we administered glucagon-like-peptide-1 (7–36) amide (GLP-1), a potent inducer of anorexia when given intracerebroventricularly^{6–9}. The dose of GLP-1 (10 µg i3vt) that we selected

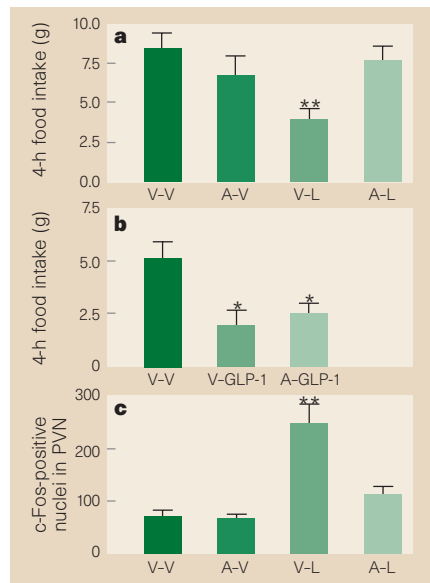


Figure 1 Effect of treatment with a melanocortin receptor antagonist on feeding and the leptin response (means±s.e.m.). V, vehicle; A, antagonist; L, leptin. Data were analysed by a one-way ANOVA followed by post-hoc Neuman-Keul's tests. * Significant difference from the vehicle-only group at $P < 0.05$; ** at $P < 0.01$. **a**, Effect of pretreatment with i3vt SHU9119 (0.5 nmol) on the anorexic response to i3vt leptin (3.5 µg) on 4-h food intake. Leptin treatments, $n = 12$; vehicle treatments, $n = 6$. **b**, The effect of pretreatment with i3vt SHU9119 (0.5 nmol) on the anorexic response to i3vt GLP-1 (10 µg) on 4-h food intake. GLP-1, $n = 5$; vehicle group, $n = 4$. **c**, Effect of pretreatment with i3vt SHU9119 (0.5 nmol) on c-Fos activation in the PVN of the hypothalamus produced by i3vt leptin (3.5 µg) treatment. Vehicle-only, $n = 5$; antagonist-vehicle, $n = 4$; leptin-treated groups, $n = 6$.

reduces short-term food intake to an extent comparable to 3.5 µg leptin⁷ (−63% after 4 h) and this effect was not altered by pretreatment with 0.5 nmol SHU9119 (Fig. 1b).

We assessed c-Fos-like-immunoreactivity in the PVN after leptin treatment with or without SHU9119 pretreatment (see ref. 10 for methods). Consistent with our previous findings¹⁰, leptin increased c-Fos-like immunoreactivity in the PVN by 254%, and this was completely blocked by SHU9119 (Fig. 1c).

Our findings provide direct evidence that MC4 receptor signalling is important in mediating leptin's effect on food intake and body weight. We suggest that obesity stemming from disrupted MC4 receptor signalling (for example, MC4-R knockout and *agouti* mice) results from the loss of an important downstream target in the leptin signalling cascade. The possibility that the melanocortin system is one mediator of leptin's action in the central nervous system is particularly intriguing in light of the recent finding that human obesity and hyperleptinaemia are strongly linked to a region of chromosome 2 near the POMC gene locus¹¹. The hypothesis that polymorphisms of the POMC gene are associated with leptin resistance and obesity is consistent with our findings implicating melanocortins in the leptin signalling pathway, and may have important implications for the pathogenesis and treatment of human obesity.

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Transcriptional squelching re-examined

The introduction of a potent transcriptional activator into eukaryotic cells can paradoxically suppress the transcription of a co-introduced target gene^{1–3}. This so-called 'squelching' is thought to result from titra-

tion of one or more general transcription factors (GTFs)⁴, indicating that GTFs might be in limited supply within the cell^{5,6}. We now find that in mammalian cells, squelching is limited to episomal target genes, whereas genes integrated into cellular chromatin are immune.

During the process of designing more potent transcriptional activation domains for use in gene therapy, we constructed chi-

maeric transcription factors composed of the yeast GAL4 DNA-binding domain and activation domains derived from the herpes simplex virus protein VP16⁷ and the NF-κB p65 protein⁸. We tested the proteins on a target gene composed of a secreted alkaline phosphatase (SEAP) reporter under the control of a minimal human interleukin-2 (IL-2) gene promoter flanked by five GAL4 binding sites.

When we transiently introduced the